



Cytogenetics of Salivary Gland Neoplasms

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Introduction

Molecular cytogenetics involves, among other things, the study of chromosomal abnormalities at the level of DNA. Chromosome translocations are genetic abnormalities caused by the transfer of a chromosomal segment to a new position, especially on a different chromosome. Translocations can result in the overexpression of one gene by placing it next to a routinely expressed gene. A common scenario in tumorigenesis is the fusion of a proto-oncogene with a promoter gene, resulting in overexpression of a functional fusion protein that leads to cell immortality. These are acquired genetic events, as opposed to congenital germline or syndrome-producing mutations. Tumor-specific chromosomal translocations have important diagnostic, prognostic and therapeutic implications and are now considered to be the defining features of a wide variety of hematogenous and soft tissue tumors.

More recently, there has been significant progress in understanding the cytogenetics of salivary gland tumors. Mucoepidermoid carcinoma and pleomorphic adenoma, the two most common salivary gland tumors¹; along with adenoid cystic carcinoma, mammary analogue secretory carcinoma and hyalinizing clear cell adenocarcinoma all harbor specific and characteristic translocations (Table 1).

Tumor	Translocation	Fusion oncogene
Adenoid cystic carcinoma	t(6;9)(q22-23;p23-24)	MYB-NFIB
Hyalinizing clear cell adenocarcinoma	t(12;22)(q13;q12)	EWSR1-ATF1
Mammary analogue secretory carcinoma	t(12;15)(p13;q25)	ETV6-NTRK3
Mucoepidermoid carcinoma	t(11;19)(q12;p13)	MECT1-MAML2
Pleomorphic adenoma	t(3;8)(p21;q12) t(5;8)(p13;q12)	CTNNB1-PLG1 LIFR-PLG1

Table 1: Salivary gland tumors and their respective translocations. Nomenclature: The "t" indicates a translocation while the first set of parentheses identifies the two chromosomes involved in the translocation, and the second set indicates the breakpoints on the chromosome arms. {O'Connor, C. Human chromosome translocations and cancer. Nature Education. 2008;1(1).}

Diagnostic Methods

A variety of molecular laboratory techniques can be employed to identify chromosomal translocations. Polymerase chain reaction involves amplification of the genetic sequence of interest using specific DNA primers.² *In situ* hybridization (ISH/FISH) utilizes paired fluorescent-tagged DNA primers that bind to both contributors of a fused gene. Both techniques can be applied to paraffin-embedded biopsies but ISH can be used directly on a microscopic slide. It is rapidly becoming the standard technique for identifying translocations.²

Adenoid Cystic Carcinoma

Adenoid cystic carcinoma (ACC) is a malignant salivary gland tumor with a propensity for perineural invasion, a female predilection and a peak incidence in the sixth decade.¹ It is not uncommon and most often involves the palate or parotid gland.¹ The prognosis is typically good for the first five years, however,

persistent growth and late metastases result in a poor 10-20 year survival rate.¹

Recently, a t(6;9)(q22-23;p23-24) translocation was identified in a subset of adenoid cystic carcinomas.³ This translocation corresponds to a gene fusion event between *NFIB*, a transcription factor³ and *MYB*, a potent oncogene identified in malignancies of pancreas, breast, and hematopoietic tissues (leukemia).⁴ The result is dramatically increased expression of *MYB*.⁴ High *MYB* expression has been correlated with poorer survival in ACC patients.⁵ At this time, assured surgical excision, close monitoring and radiation therapy as required are the recommended therapy for isolated tumors¹, however, targeting downstream *MYB-NFIB* products and proposed DNA vaccines against the *MYB* gene segment may provide future therapeutic approaches.⁴

Hyalinizing Clear Cell Adenocarcinoma

A relatively rare tumor, hyalinizing clear cell adenocarcinoma (HCCA) is a low grade malignancy which primarily occurs in the minor salivary glands of older males and females.¹ Histologically, it is composed of cords and nests of clear epithelial cells embedded in a hyalinized stroma.⁶

Current evidence indicates that a t(12;22)(q13;q12) gene translocation differentiates HCCA from other clear cell tumors such as mucoepidermoid carcinoma, myoepithelial carcinoma and epithelial-myoepithelial carcinoma.⁶ Interestingly, a relationship between HCCA and clear cell odontogenic carcinoma has recently been described. The t(12;22) translocation was identified in 82% of HCCA cases and 83% of clear cell odontogenic carcinoma cases, suggesting they may represent a central variant of HCCA.^{7,8}

The t(12;22) translocation results in an *EWSR1-ATF1* fusion that is also present in clear cell sarcomas and angiomatoid fibrous histiocytomas.⁶ *EWSR1* encodes a multifunctional protein that is involved in various cellular processes, including gene expression, cell signaling and RNA processing. It is notable for its tumorigenic role in Ewing sarcoma and related tumors.⁹

Mammary Analogue Secretory Carcinoma

Mammary analogue secretory carcinoma (MASC) of salivary glands was first described in 2010 by Skálová and colleagues after identifying 16 salivary gland tumors that had previously been categorized as acinic cell adenocarcinomas or cystadenocarcinomas.¹⁰ Microscopically, the tumors resemble secretory carcinoma of the breast and demonstrate a lobulated growth pattern with microcystic and glandular spaces filled by eosinophilic secretory product.¹⁰ They occur primarily in the parotid gland with a slight male predilection and a mean age of 46 years at time of presentation.^{10,11}

MASC harbors a t(12;15)(p13;q25) translocation, which results in the *ETV6-NTRK3* fusion oncogene¹⁰ encoding a chimeric tyrosine kinase, that has also been identified in secretory breast carcinomas, congenital fibrosarcoma, leukemias and other malignancies.¹⁰⁻¹² Although MASC typically demonstrates clinical features and outcomes similar to the low-grade acinic cell adenocarcinoma, targeting the chimeric tyrosine kinase protein may be a future therapeutic option.¹⁰⁻¹²

Mucoepidermoid Carcinoma

The most common malignant salivary gland tumor in both adults and children is the mucoepidermoid carcinoma (MEC), which occurs primarily in parotid gland and is composed of admixed mucocytes, epidermoid cells and intermediate cells forming cysts and nests.¹

A t(11;19)(q12;p13) translocation that pairs *MECT1* and *MAML2* has been identified as an underlying event in the majority of MECs.³ The *MECT1-MAML2* chimeric (fusion) protein is thought to cause disruption of cell cycle and differentiation functions through dysregulation of c-AMP response element-binding (CREB), resulting in tumorigenesis and increased activation of Notch signaling pathways.¹³ The presence of this genetic event in MECs is associated with a lower clinical stage and an overall more favorable outcome.¹⁴

Pleomorphic Adenoma

Pleomorphic adenoma is a benign salivary gland tumor composed of proliferating ductal and myoepithelial elements in a variably myxoid to chondroid background. An infinite number of architectural configurations can be seen.¹ It is by far the most common salivary gland tumor and primarily involves the parotid and minor glands.¹ Although benign, these tumors have a propensity for recurrence if not completely excised.¹

Pleomorphic adenomas are also characterized by recurrent chromosomal rearrangements, most commonly t(3;8)(p21;q12)¹⁵ which results in promoter swapping between *PLG1*, a novel developmentally-regulated zinc finger gene¹⁵ recently characterized as a proto-oncogene¹⁶, and *CTNNB1* which encodes for β -catenin.¹⁵ The activation of *PLG1* is considered a frequent genetic event occurring in all major cytogenetic subgroups of pleomorphic adenomas, and is also demonstrated in certain mesenchymal tumors.¹⁷

PLG1 is also involved in the second most common translocation of pleomorphic adenomas, t(5;8)(p13;q12) which again results in increased expression of *PLG1*, except under control of the *LIFR* (leukemia inhibitory factor) promoter region, which is active in a wide variety of fetal and adult tissues, including normal salivary gland tissue.¹⁸ This information has helped reinforce the theory that pleomorphic adenoma cells originate from a single pluripotent cell type capable of differentiation into a variety of somatic phenotypes.¹⁹

References

1. Ellis GL, Auclair PL. Tumors of the Salivary Glands. Atlas of Tumor Pathology, 4th series, Fascicle 9, 1 ed. Washington, D.C.: Armed Forces Institute of Pathology, 2007.
2. Jordan RC, Daniels TE, Greenspan JS, Regezi JA. Advanced diagnostic methods in oral and maxillofacial pathology. Part I: Molecular methods. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2001;92(6):650-669.
3. Persson M, Andren Y, Mark J, Horlings HM, Persson F, Stenman G. Recurrent fusion of MYB and NFIB transcription factor genes in carcinomas of the breast and head and neck. Proc Natl Acad Sci U S A. 2009;106:18740-4.
4. Bell D, Hanna, EY. Salivary gland cancers: Biology and molecular targets for therapy. Curr Oncol Rep. 2012;14:166-174.
5. Mitani Y, Rao PH, Futreal PA, Roberts DB, Stephens PJ, Zhao YJ, Zhang L, Mitani M, Weber RS, Lippman SM, Caulin C, El-Naggar AK. Novel chromosomal rearrangements and break points at the t(6;9) in salivary adenoid cystic carcinoma: Association with MYB-NFIB chimeric fusion, MYB expression, and clinical outcome. Clin Cancer Res. 2011;17(22):7003-14.
6. Tanguay K, Weinreb I. What the EWSR1-ATF1 fusion has taught us about hyalinizing clear cell carcinoma. Head and Neck Pathol. 2013;7:28-34.
7. Bilodeau EA, Weinreb I, Antonescu CR, Zhang L, Dacic S, Muller S, Barker B, Seethala RR. Clear cell odontogenic carcinomas show EWSR1 rearrangements: a novel finding & biologic link to salivary clear cell carcinomas. Mod Pathol. 2012;25 (Supplement 2s):101:305A.
8. Bilodeau EA, Hoschar AP, Barnes EL, Hunt JL, Seethala RR. Clear cell carcinoma and clear cell odontogenic carcinoma: a comparative clinicopathologic and immunohistochemical study. Head Neck Pathol. 2011;5(2):101-7.
9. Li H, Watford W, Li C, Parmelee A, Bryant MA, Deng C, O'Shea J, Lee SB. Ewing sarcoma gene EWS is essential for meiosis and B lymphocyte development. J Clin Invest. 2007;117:1314-1323.
10. Skálová A, Vanecek T, Sima R, Laco J, Weinreb I, Perez-Ordóñez B, Starek I, Geierova M, Simpson RH, Passador-Santos F et al. Mammary analogue secretory carcinoma of salivary glands, containing the ETV6-NTRK3 fusion gene: a hitherto undescribed salivary gland tumor entity. Am J Surg Pathol. 2010;34(5):599-608.
11. Bishop, JA. Unmasking MASC: Bringing to Light the Unique Morphologic, Immunohistochemical and Genetic Features of the Newly Recognized Mammary Analogue Secretory Carcinoma of Salivary Glands. Head and Neck Pathol. 2013;7:35-39.
12. Chiosea SI, Griffith C, Assaad A, Seethala RR. Clinicopathological characterization of mammary analogue secretory carcinoma of salivary glands. Histopathology. 2012;61:387-94.
13. Tonon G, Modi S, Wu L, Kubo A, Coxon AB, Komiya T, O'Neil K, Stover K, El-Naggar A, Griffin JD, Kirsch IR, Kaye FJ. t(11;19)(q21;p13) translocation in mucoepidermoid carcinoma creates a novel fusion product that disrupts a Notch signaling pathway. Nat Genet. 2003;33(2):208-13.
14. Okabe M, Miyabe S, Nagatsuka H, Terada A, Hanai N, Yokoi M, Shimozato K, Eimoto T, Nakamura S, Nagai N, Hasegawa Y, Inagaki H. MECT1-MAML2 fusion transcript defines a favorable subset of mucoepidermoid carcinoma. Clin Cancer Res. 2006;12:3902-3907.
15. Kas K, Voz ML, Röijer E, Aström AK, Meyen E, Stenman G, Van de Ven WJ. Promoter swapping between the genes for a novel zinc finger protein and beta-catenin in pleomorphic adenomas with t(3;8)(p21;q12) translocations. Nature Genet 1997;15(2):170-174.
16. Hensen K, Van Valckenborgh IC, Kas K, et al. The tumorigenic diversity of the three PLAG family members is associated with different DNA binding capacities. Cancer Res 2002;62:1510-1517.
17. Aström AK, Voz ML, Röijer E, Wedell B, Mandahl N, Van de Ven W, Mark J, Stenman G. Conserved mechanism of PLAG1 activation in salivary gland tumors with and without 8q12 abnormalities: identification of SHI as a new fusion pleomorphic adenoma partner gene. Cancer Res 1999;59:918-923.
18. Voz ML, Aström AK, Kas K, Mark J, Stenman G, Van de Ven W. The recurrent translocation t(5;8)(p13;q12) in pleomorphic adenomas results in upregulation of PLAG1 gene expression under control of the LIFR promoter. Oncogene. 1998;16:1409-1416.
19. Martins C, Fonseca I, Roque L, Pereira T, Ribeiro C, Bullerdiek J, Soares J. PLAG1 gene alterations in salivary gland pleomorphic adenoma and carcinoma ex-pleomorphic adenoma: a combined study using chromosome banding, in situ hybridization, and immunohistochemistry. Modern Pathology. 2005;18:1048-1055.

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